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Coordinated methyl and RNA binding is required for heterochromatin localization of mammalian HP1α

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In mammalian cells, as in *Schizosaccharomyces pombe* and *Drosophila*, HP1 proteins bind histone H3 tails methylated on lysine 9 (K9). However, whereas K9-methylated H3 histones are distributed throughout the nucleus, HP1 proteins are enriched in pericentromeric heterochromatin. This observation suggests that the methyl-binding property of HP1 may not be sufficient for its heterochromatin targeting. We show that the association of HP1α with pericentromeric heterochromatin depends not only on its methyl-binding chromo domain but also on an RNA-binding activity present in the hinge region of the protein that connects the conserved chromo and chromoshadow domains. Our data suggest the existence of complex heterochromatin binding sites composed of methylated histone H3 tails and RNA, with each being recognized by a separate domain of HP1α.

INTRODUCTION

HP1 proteins are non-histone constituents of chromatin, preferentially located in heterochromatin and associated with transcriptional repression (Eissenberg and Elgin, 2000). HP1 proteins are well conserved through evolution, as homologues exist in *Schizosaccharomyces pombe*, *Drosophila* and mammals. They all contain two weakly homologous motifs known as the chromo domain and the chromoshadow domain, located in the N-terminal and C-terminal regions of the protein, respectively. The chromo domain of HP1 proteins can associate with histone H3 tails when methylated on the lysine at position 9 (MetK9 H3 histones). This high-affinity binding activity has been well characterized (Bannister *et al.*, 2001; Jacobs *et al.*, 2001; Lachner *et al.*, 2001; Nielsen *et al.*, 2002). However, MetK9 H3 histones are found throughout the nucleus and are not restricted to heterochromatin (Peters *et al.*, 2001; Maison *et al.*, 2002), suggesting that the methyl-binding activity of HP1 proteins is not sufficient for their targeting to inactive chromatin.

In mouse cells, HP1 proteins concentrate in the foci of highly condensed pericentromeric heterochromatin. Interestingly, these foci are specifically detected by antibodies recognizing four MetK9 H3 peptides organized in a branched configuration. It was therefore suggested that, within mouse pericentromeric heterochromatin, methylated histone H3 tails are assembled in particular three-dimensional structures. Surprisingly, treatment of the cells with RNase causes dispersion of HP1 proteins from the pericentomeric foci and prevents detection of the foci by anti-branched MetK9 H3 antibodies. This observation further suggested that the structures recognized by the antibodies and HP1 also contain an RNA component (Peters *et al.*, 2001; Maison *et al.*, 2002). In the present study, we investigate the mechanisms allowing HP1α to specifically target pericentromeric heterochromatin. We demonstrate that the methyl-binding activity of this protein is not sufficient for detection of its chromatin binding sites and that the chromo domain must be supplemented by an RNA-binding activity, located in a neighbouring protein domain, to achieve proper sub-nuclear localization.

RESULTS

Purified recombinant HP1α binds to nuclear structures in a fashion similar to endogenous HP1α

Recently, several studies (Nielsen *et al.*, 2001; Taddei *et al.*, 2001; Maison *et al.*, 2002) have used far-western-type overlay

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assays for the characterization of HP1 proteins (Figure 1A). To further substantiate the validity of this procedure, nuclear extracts from mouse NIH 3T3 fibroblasts were resolved by SDS–PAGE, transferred to nitrocellulose and incubated with either GST or GST–HP1α fusion protein produced in *Escherichia coli*. GST–HP1α, but not GST alone, strongly bound to a single species migrating in the position of histone H3 (Figure 1B, lanes 2 and 3). To confirm specificity of HP1α binding in this assay, we resolved a purified calf thymus core histone preparation and challenged binding with a peptide corresponding to the first 17 amino acids of histone H3 either unmethylated or methylated on K9. HP1α bound to a protein co-migrating with histone H3 (Figure 1C, lane 2), and this binding was efficiently competed with only methylated histone H3 peptide (cf. lanes 3 and 4 in Figure 1C). HP1α histone binding was also challenged with RNase A, which had no effect on HP1α affinity for histone H3 (Figure 1C, lane 5).

Overlay assays were also performed on paraformaldehyde-fixed NIH 3T3 cells. Incubation of fixed and permeabilized cells with recombinant GST–HP1α followed by antibody staining provided a pattern similar to that observed with anti-HP1α antibodies, with a clear preference for the foci of pericentromeric heterochromatin that appear as large dots upon staining with DAPI (Figure 1D, E and I). As above, binding was efficiently competed with methylated H3 peptide but not with unmodified peptide (Figure 1F and G). As mentioned in the Introduction, the treatment of mouse fibroblasts with RNase before fixation causes HP1α to de-localize from the foci of pericentromeric heterochromatin (Maison *et al.*, 2002). To verify that RNase would also affect HP1α localization in overlay assays, GST–HP1α incubation was performed in the presence of RNase A. As *in vivo*, recombinant HP1α failed to bind pericentromeric heterochromatin in the absence of RNA (Figure 1H). However, we noted a persistant diffuse nuclear signal with some concentration in the nucleoli, identified as regions weakly stained by DAPI.

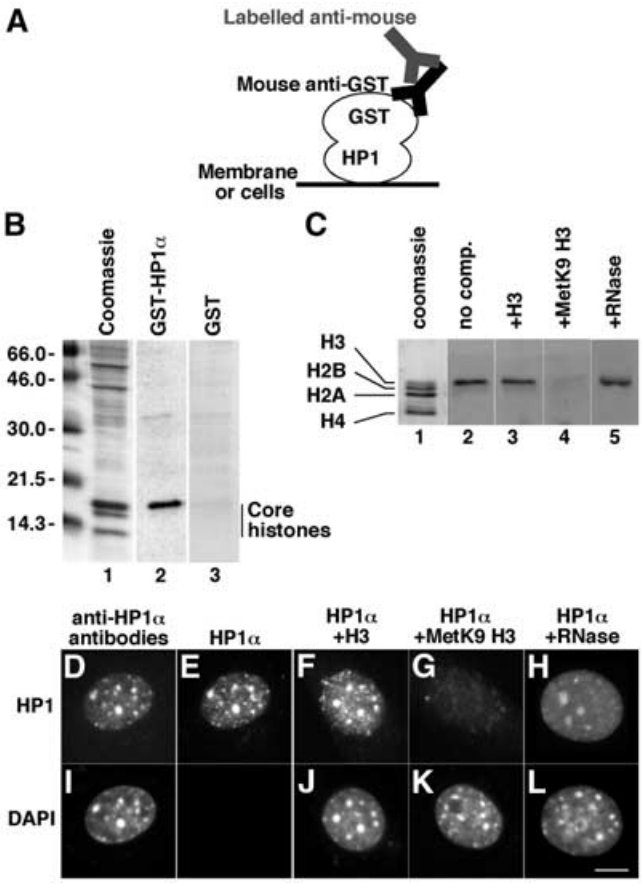

Fig. 1. HP1α specifically binds methylated histone H3 tails in overlay assays. (**A**) Schematic representation of the far-western-type overlay assays. Recombinant GST–HP1 fusion proteins produced in *E. coli* were incubated with western-blotted membranes or paraformaldehyde-fixed cells. After washing, retained GST–HP1 was detected using anti-GST antibodies produced in mouse and then labelled anti-mouse antibodies. (**B**) Nuclear extracts were resolved by SDS–PAGE and either stained with Coomassie Blue (lane 1) or transferred to nitrocellulose. The membrane was cut to separate the lanes, and each lane was incubated with either GST–HP1α (lane 2) or GST (lane 3). Size markers are indicated (in kDa). (**C**) Purified histones were resolved by SDS–PAGE and treated as in (A). Each individual lane was incubated with GST–HP1α either in the absence of competing peptide (lane 2) or in the presence of either unmethylated (lane 3), tri-methylated H3 peptide (lane 4) or RNase A (lane 5). Lane 1 shows Coomassie Blue staining of the gel. (**D**, **E** and **I**) Fixed NIH 3T3 cells were incubated with anti-HP1α antibodies and then washed and incubated with recombinant GST–HP1α fusion protein as described in (A). (**F**, **G**, **J** and **K**) Competition experiments were performed by incubating cells with GST–HP1α and the same peptides used in (C). (**H** and **L**) Cells were incubated simultaneously with GST–HP1α and RNase A. Scale bar: 6 μm.

Binding specificity of HP1α is conferred by the chromo domain and the hinge region

The experiments shown above indicated that binding of recombinant HP1α to nuclear structures was, like endogenous HP1α, dependent on the presence of both methylated histone H3 tails and RNA. Therefore, we used the overlay technique to identify the domains of HP1α responsible for this targeting. Several mutant GST–HP1α fusion proteins were expressed in *E. coli*, affinity purified and tested for their ability to bind heterochromatin foci (Figure 2A). These, or similar fusion proteins, have been shown to bind purified histones on membranes only when containing an intact chromo domain (Nielsen *et al.*, 2001). As a control for our mutant protein preparations, overlay assays on purified histones are also shown in Figure 2B. On cells, a double point mutation in the chromo domain led to de-localized HP1α staining. Besides, neither the chromo domain, the chromoshadow domain nor the hinge region taken individually could target the HP1α proteins to heterochromatin (Figure 2D, F, G and H). Conversely, a construct containing both an intact chromo domain and sequences from the hinge region [HP1α(1–119)] displayed a dotted nuclear binding pattern similar to wild-type HP1α (cf. Figure 2C and E). These observations suggest that the presence of both the chromo domain and the hinge region are required for targeting of HP1α to pericentromeric foci.

A conserved region of the hinge is required for proper HP1α localization

The hinge region is less well conserved among HP1 proteins than the chromo and chromoshadow domains. However, the C-terminal part of this region contains several charged amino

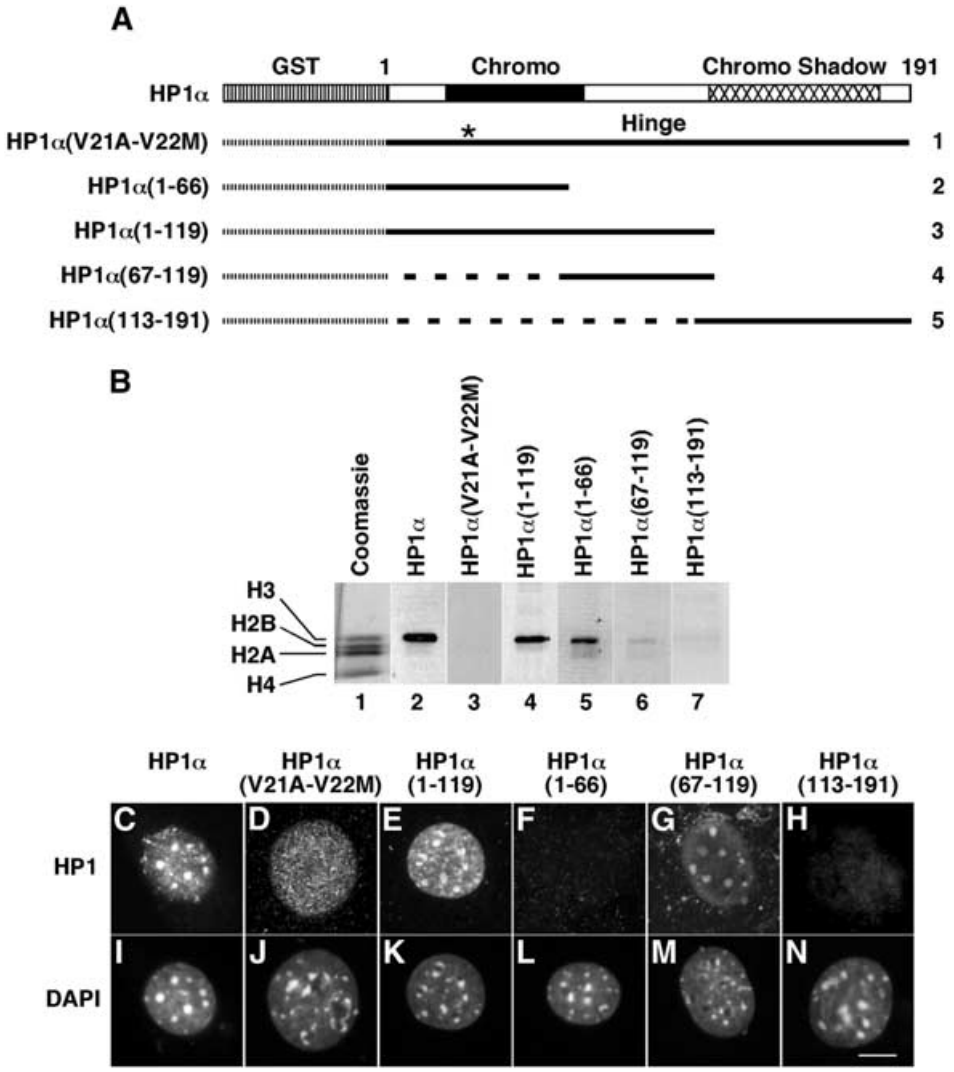

Fig. 2. Binding of HP1α to pericentromeric heterochromatin requires both the chromo domain and the hinge. (**A**) Schematic representation of the HP1α deletion constructs. (**B**) Purified histones were resolved by SDS–PAGE as in Figure 1C and then incubated with the indicated GST–HP1α constructs as described in Figure 1A. (**C**–**N**) Fixed NIH 3T3 cells were incubated with indicated GST–HP1α constructs as described in Figure 1A. Dots observed upon staining with HP1α(67–119) correspond to the nucleoli. DNA was stained with DAPI (I–N). Scale bar: 6 μm.

acids present in all three mammalian HP1-family members (Figure 3A). A homology of this region with the DNA-binding domain of human centromere protein C (CENP-C) has also been suggested (Sugimoto *et al.*, 1996). In overlay assays on fixed cells, mutation of three consecutive lysines (K104, K105 and K106), contained within this conserved region, abolished the ability of HP1α to concentrate in pericentromeric foci [HP1α(3 × K→A), Figure 3C]. Inversely, a 34-amino-acid sequence, centred on these lysines, was targeted to pericentromeric heterochromatin when fused to an intact chromo domain [cf. the binding of HP1α(1–72) and HP1α(1–72)+(86–119), Figure 3D and E]. To further characterize the 34-amino-acid domain, we tested two additional constructs containing an intact chromo domain, the lysine-rich region and residues located either immediately upstream [HP1α(1–72)+(86–108)] or downstream [HP1α(1–72) +(99–119)] of the lysines. Of these constructs, only HP1α(1–72) +(86–108) was targeted to pericentromeric foci (cf. Figure 3F and G). We conclude from these experiments that pericentromeric localization of HP1α requires the concomitant effects of the chromo domain and amino acids 86–108 of the hinge region.

HP1α is an RNA-binding protein

HP1α and *Drosophila* HP1a have been reported to bind DNA, and the binding domain has been mapped to the hinge region (Sugimoto *et al.*, 1996; Zhao *et al.*, 2000). Because RNase could de-localize HP1α from pericentromeric heterochromatin, we investigated whether HP1α would also bind RNA. Electrophoretic mobility shift assays (EMSAs) were performed with radioactively labelled RNA transcribed from a randomly chosen bacterial sequence. In these assays, HP1α efficiently bound the RNA probe (Figure 4A, lane 2). This binding was efficiently competed with unlabelled probe RNA (Figure 4A, lanes 3 and 4) but resisted competition with up to 6000-fold molar excess of either

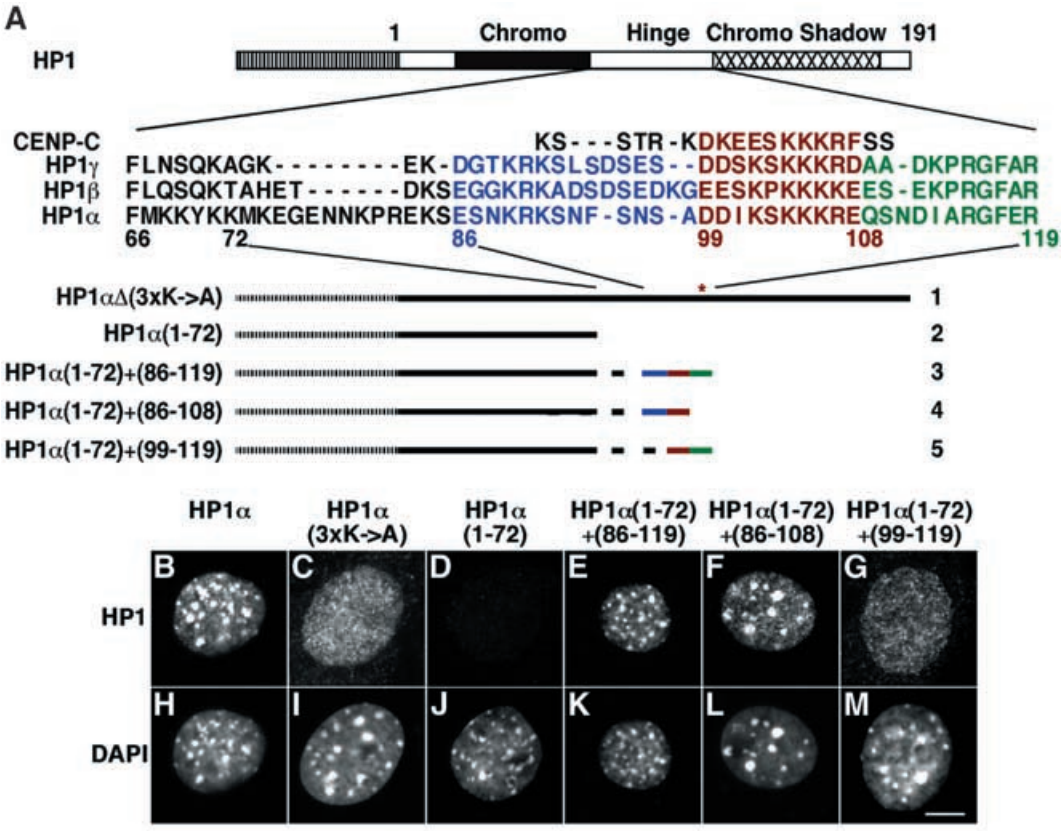

Fig. 3. A conserved region of the hinge is required for pericentromeric binding. (**A**) Amino acid alignment of the hinge region present in human HP1α, HP1β and HP1γ, part of the suggested alignment with human centromere protein C (CENP-C; Sugimoto *et al.*, 1996), and a schematic representation of the HP1α deletion constructs. (**B**–**M**) Fixed NIH 3T3 cells were incubated with indicated GST–HP1α constructs as described in Figure 1A. DNA was stained with DAPI (H–M). Scale bar: 6 μm.

single- or double-stranded DNA with the same sequence as the RNA probe (Figure 4A, lanes 5–10). Binding resisted competition with 3000-fold molar excess of AU- or GC-rich 20-mer oligoribonucleotides (Figure 4B, lanes 9–12). Poor competition was also observed with tRNA, whereas rRNA or nuclear RNA were more efficient (Figure 4B, lanes 3–8). In fact, nuclear RNA was a more efficient competitor than unlabelled probe RNA (Figure 4B, lanes 14–20). Various HP1α mutants were also tested for RNA binding. In this assay, only constructs containing amino acids 86–108 were associating with the RNA probe (Figure 4C, lanes 1–11, 15 and 16). Inversely, mutation of K104, K105 and K106 abolished the RNA-binding activity (Figure 4C, lane 14).

Divergent RNA-binding properties of HP1α and HP1γ

Mammalian HP1γ, another member of the HP1 family of proteins, is frequently described as being more diffusely distributed than HP1α (Minc *et al.*, 2000; Nielsen *et al.*, 2001), and a larger fraction of this protein seems to be localized outside the pericentromeric foci, when compared to HP1α (cf. Figure 5A and B). In overlay assays, HP1γ concentrates in pericentromeric foci like HP1α. Besides, like HP1α, treatment of cells with RNase before fixation causes de-localization of HP1γ (Maison *et al.*, 2002; cf. Figure 5D–G). However, alignment of the sequences of HP1α and HP1γ revealed some differences with the region required for RNA binding of HP1α. Therefore, we tested RNA-binding activity of HP1γ expressed in *E. coli* either as a GST fusion protein or tagged with six histidines. In contrast to HP1α, neither of the two HP1γ constructs bound our randomly chosen RNA probe in EMSAs (Figure 5L). To further characterize the RNA-binding properties of HP1γ, we used a north-western-type assay in which proteins were resolved by SDS–PAGE, transferred to nitrocellulose, re-natured and finally incubated with the labelled RNA probe. Unlike GST or HP1α mutated in K104, K105 and K106, wild-type HP1α bound the RNA probe in this assay (Figure 5M, lanes 1–5 and 9–11). Unlike the mobility-shift assays, the north-western assays revealed RNA binding of HP1γ, but with a reduced avidity for the RNA probe compared with HP1α (Figure 5M, lanes 6–8). These observations suggest that, although both HP1α and HP1γ are RNA-binding proteins, they may display some differences in their binding properties.

DISCUSSION

Domains of HP1α required for chromatin binding

Recently, the *Drosophila* chromo domain proteins MOF and MSL-3, involved in male X-chromosome dosage compensation,

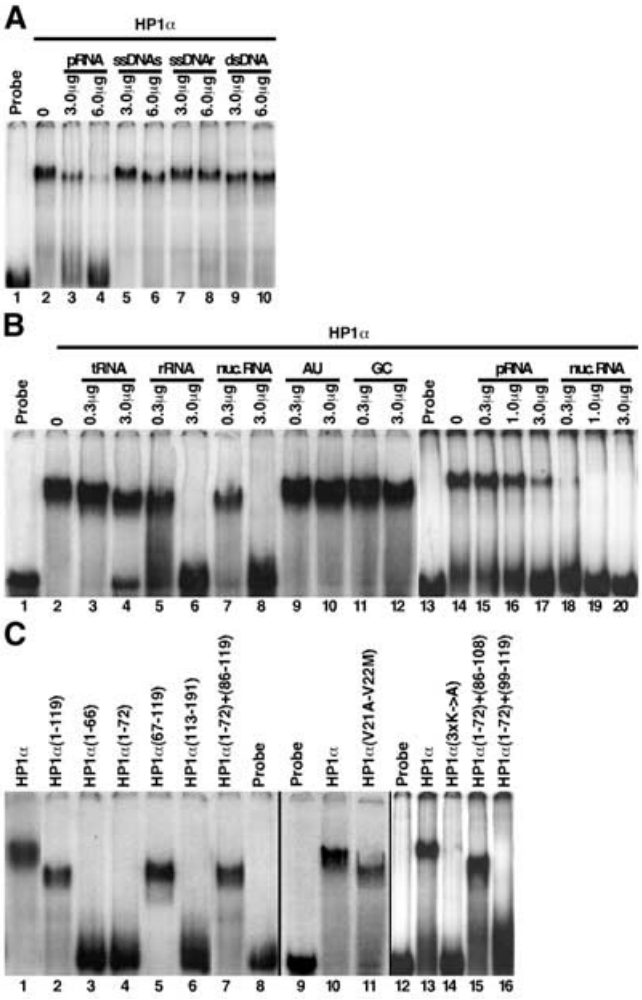

Fig. 4. HP1α is an RNA-binding protein. (**A**) Approximately 1 μg of GST–HP1α (lanes 2–6) was incubated in the presence of a radioactively labelled RNA probe. Incubation was performed either in the absence (lane 2) or in the presence of either unlabelled probe RNA (lanes 3 and 4), single-stranded DNA with the same sequence as the RNA probe (lanes 5 and 6) or the anti-parallel sequence (lanes 7 and 8), or double-stranded DNA with the same sequence (lanes 9 and 10). (**B**) As in (A), 1 μg of GST–HP1α (lanes 2–12 and 14–20) was incubated in the presence of a radioactively labelled RNA probe. Incubation was performed either in the absence (lanes 2 and 14) or in the presence of tRNA (lanes 3 and 4), rRNA (lanes 5 and 6), nuclear RNA (lanes 7 and 8 and 18–20), AU_{10} (lanes 9 and 10) and GC_{10} (lanes 11 and 12) oligoribonucleotides, or unlabelled probe RNA (lanes 15–17) in the amounts indicated. (**C**) EMSAs were performed as in (A), using the indicated GST–HP1α fusion proteins.

were shown to bind RNA and interact with the X chromosome in an RNase-sensitive manner (Akhtar *et al.*, 2000). As revealed here by EMSAs, HP1α is also an RNA-binding protein. However, unlike MOF, HP1α RNA binding is not mediated by the chromo domain itself but by a 23-amino-acid domain located in the C-terminal part of the neighbouring hinge region. This domain shows no obvious homology to known RNA-binding proteins, although domains rich in lysines and arginines are known to contact RNA in several ribosomal proteins as well as in retroviral virulence factors such as HIV TAT (Weiss and Narayana, 1998; Brodersen *et al.*, 2002). Overlay assays on fixed cells showed that both the chromo domain and the hinge were required for targeting of HP1α to pericentromeric heterochromatin. Indeed, the chromo domain and the hinge appear to function as independent modules, acting as either methyl- or RNA-binding units, respectively. As illustrated with the HP1α(1–72)+(86–108) construct, which fuses the chromo domain to a portion of the hinge, HP1α targeting to heterochromatin foci could be achieved by a simple combination of these two modules, without regard for the initial spacing. Such a modular binding mechanism suggests a large degree of flexibility in the relative positioning of the methylated histone tails and the RNA within the HP1 binding sites.

RNA-binding properties of HP1 proteins

In EMSAs, HP1α showed a clear preference for RNA rather than DNA. In addition, nuclear RNA was more efficient in competition experiments than tRNA or our randomly chosen probe RNA, suggesting some degree of specificity in the binding. It must be noted that MOF and MSL-3 show no or little binding specificity *in vitro*, although their physiological target is roX RNA (Akhtar *et al.*, 2000). Further studies will be required to determine whether HP1α binds single or multiple RNA molecules *in vivo*. Interestingly, ribosomal RNA preparations were also very efficient in competition experiments. Since pericentromeric heterochromatin is frequently juxtaposed to the nucleoli (Akhmanova *et al.*, 2000), it is tempting to speculate that nucleolar RNA species may participate in the structure of heterochromatin.

Surprisingly, we found that HP1γ, another member of the HP1 family, failed to bind our RNA probe in EMSAs, although this protein was, like HP1α, de-localized from the pericentromeric foci by RNase treatment. Further studies revealed that HP1γ could in fact bind RNA in the more gentle north-western assays. *In vivo*, binding of HP1γ to RNA may be dependent on the correct display of the target within a chromatin structure. Alternatively, HP1γ RNA binding may be more sequence-specific than that of HP1α, possibly explaining the differences in the sub-nuclear localization of HP1α and HP1γ.

Taken together with the recent observations by Maison *et al.* (2002), our data clearly demonstrate that targeting of HP1α to pericentromeric heterochromatin depends on both specific configurations of modified histone tails and RNA components. Our experiments also add further support to the involvement of RNA in silencing beyond inactivation of the X chromosome.

METHODS

Plasmids and recombinant GST fusions. Expression vectors derived from pGEX for GST–HP1α(1–119), GST–HP1α(1–66) and GST–HP1α(67–119) were kindly provided by R. Losson (Nielsen *et al.*, 2001). GST–HP1α, GST–HP1α(113–191) and GST–HP1γ were described previously (Seeler *et al.*, 1998). All constructs were expressed in *E. coli* strain BL21 and purified according the recommendations of the glutathione–Sepharose manufacturer (Amersham Pharmacia Biotech).

Far-western assays. Extracts from NIH 3T3 cells lysed in 8 M urea (30 μg per lane) or acid extracted histones from calf thymus (1 μg per lane) were resolved by SDS–PAGE and transferred to nitrocellulose membranes. Each lane was then cut and

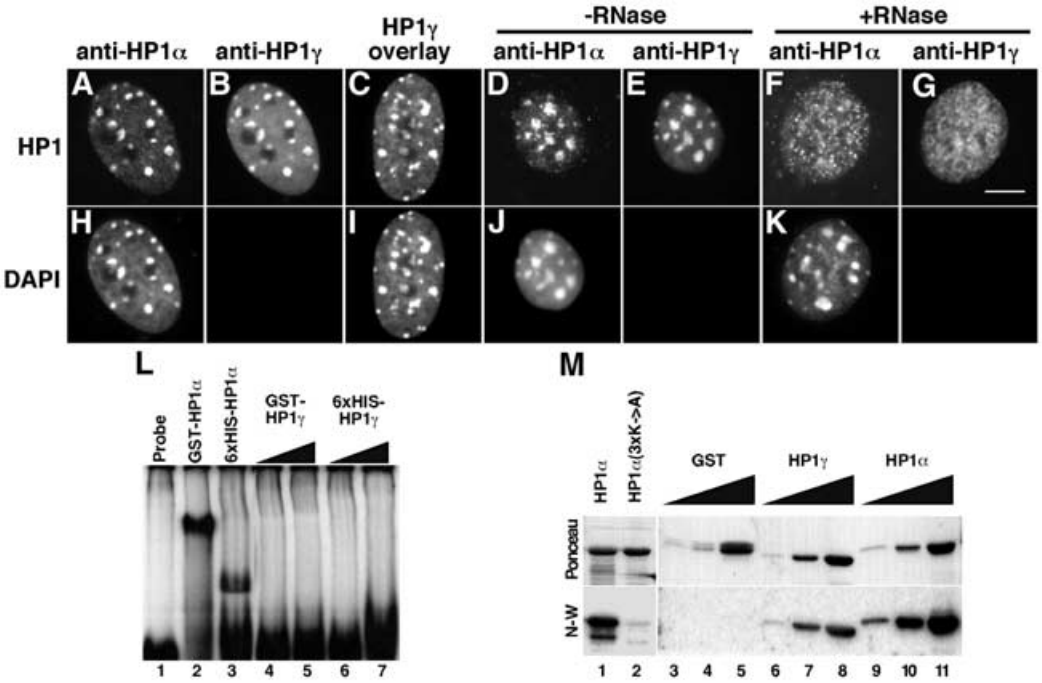

Fig. 5. Divergent RNA-binding properties of HP1γ. (**A**–**C**) Paraformaldehyde-fixed NIH 3T3 cells were indirectly stained with anti-HP1α, anti-HP1γ or GST–HP1γ as indicated. (**D**–**G**) NIH 3T3 cells were permeabilized with Triton X-100 and then incubated either in the absence (D and E) or in the presence of RNase A (F and G). Cells were then fixed and indirectly stained with anti-HP1α and anti-HP1γ antibodies as indicated. DNA was stained with DAPI (**H**–**K**). Scale bar: 6 μm. (**L**) Approximately 1 μg of the indicated HP1α constructs (lanes 2 and 3) or 1 and 3 μg of the indicated HP1γ constructs were incubated in the presence of a randomly chosen radioactively labelled RNA probe. (**M**) North-western assays. Either GST–HP1α (lane 1), GST–HP1α(3 × K→A) (lane 2), GST (lanes 3–5), 6×HIS-HP1γ (lanes 6–8) or 6×HIS-HP1α (lanes 9–11) were resolved by SDS–PAGE, transferred to nitrocellulose, re-natured and then incubated with the radioactively labelled RNA probe.

incubated overnight with 0.6 μg/ml recombinant GST–HP1α or GST–HP1γ fusion proteins in PBS-1% Tween-5% BSA either in the absence or in the presence of 100-fold molar excess of histone H3 peptide. Next, membranes were incubated with anti-GST monoclonal antibodies as for normal western blots. The sequence of the H3 peptide used is ARTKQTARKSTGGKAPR. The methylated peptide was tri-methylated on K9.

Immunocytochemistry. NIH 3T3 cells were paraformaldehyde fixed and permeabilized with PBS-0.3% Triton X-100. Coverslips were incubated overnight in the presence of GST–HP1 fusion proteins as for the far-western assays. Next, cells were incubated with mouse anti-GST and other indicated antibodies and then with secondary antibodies before final staining with DAPI. Extraction of live cells: cells grown on coverslips incubated for 3 min on ice in PNS-0.3% Triton X-100 (PNS buffer contains 20 mM PIPES pH 6.8, 200 mM NaCl, 600 mM sucrose). The coverslips were then incubated for 1 h on ice in PNS buffer or PNS buffer containing RNase A (1 mg/ml). Cells were fixed in PNS-3.7% paraformaldehyde then treated as above. Monoclonal anti-HP1α (1H5) or HP1γ (1G6) were purchased from Euromedex. Monoclonal anti-GST antibodies were a gift from O. Jeannequin and J.L. Guesdon. Polyclonal anti-HP1α antibodies were raised against amino acids 1–119.

RNA-binding assays. EMSAs were performed in RNA Binding Buffer (20 mM HEPES pH 7.6, 100 mM KCl, 2 mM EDTA, 0.01% NP40). The probe was produced by linearizing pGEM7 with *Eco*RI and then *in vitro* transcribing the template using T7 RNA polymerase in the presence of radio-actively labelled CTP (using a 'Riboprobe' kit from Promega). Samples were loaded on a 5% polyacrylamide gel in 0.25× TBE. For north-western assays, re-natured HP1 proteins bound to nitrocellulose membrane were incubated with labelled RNA probe for 1 h in RNA Binding Buffer and washed three times in the same buffer.

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REFERENCES

Akhmanova, A., Verkerk, T., Langeveld, A., Grosveld, F. and Galjart, N. (2000) Characterisation of transcriptionally active and inactive chromatin domains in neurons. *J. Cell Sci.*, **113**, 4463–4474.

Akhtar, A., Zink, D. and Becker, P.B. (2000) Chromodomains are protein–RNA interaction modules. *Nature*, **407**, 405–409.

Bannister, A.J., Zegerman, P., Partridge, J.F., Miska, E.A., Thomas, J.O., Allshire, R.C. and Kouzarides, T. (2001) Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain. *Nature*, **410**, 120–124.

Brodersen, D.E., Clemons, Jr, W.M., Carter, A.P., Wimberly, B.T. and Ramakrishnan, V. (2002) Crystal structure of the 30 S ribosomal subunit from *Thermus thermophilus*: structure of the proteins and their interactions with 16 S RNA. *J. Mol. Biol.*, **316**, 725–768.

Eissenberg, J.C. and Elgin, S.C. (2000) The HP1 protein family: getting a grip on chromatin. *Curr. Opin. Genet. Dev.*, **10**, 204–210.

Jacobs, S.A., Taverna, S.D., Zhang, Y., Briggs, S.D., Li, J., Eissenberg, J.C., Allis, C.D. and Khorasanizadeh, S. (2001) Specificity of the HP1 chromo domain for the methylated N-terminus of histone H3. *EMBO J.*, **20**, 5232–5241.

Lachner, M., O'Carroll, D., Rea, S., Mechtler, K. and Jenuwein, T. (2001) Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. *Nature*, **410**, 116–120.

Maison, C., Bailly, D., Peters, A.H., Quivy, J.P., Roche, D., Taddei, A., Lachner, M., Jenuwein, T. and Almouzni, G. (2002) Higher-order structure in pericentric heterochromatin involves a distinct pattern of histone modification and an RNA component. *Nat. Genet.*, **30**, 329–334.

Minc, E., Courvalin, J.C. and Buendia, B. (2000) HP1γ associates with euchromatin and heterochromatin in mammalian nuclei and chromosomes. *Cytogenet. Cell Genet.*, **90**, 279–284.

Nielsen, A.L., Oulad-Abdelghani, M., Ortiz, J.A., Remboutsika, E., Chambon, P. and Losson, R. (2001) Heterochromatin formation in mammalian cells: interaction between histones and HP1 proteins. *Mol. Cell*, **7**, 729–739.

Nielsen, P.R., Nietlispach, D., Mott, H.R., Callaghan, J., Bannister, A., Kouzarides, T., Murzin, A.G., Murzina, N.V. and Laue, E.D. (2002) Structure of the HP1 chromodomain bound to histone H3 methylated at lysine 9. *Nature*, **416**, 103–107.

Peters, A.H. *et al.* (2001) Loss of the suv39h histone methyltransferases impairs mammalian heterochromatin and genome stability. *Cell*, **107**, 323–337.

Seeler, J.S., Marchio, A., Sitterlin, D., Transy, C. and Dejean, A. (1998) Interaction of SP100 with HP1 proteins: a link between the promyelocytic leukemia-associated nuclear bodies and the chromatin compartment. *Proc. Natl Acad. Sci. USA*, **95**, 7316–7321.

Sugimoto, K., Yamada, T., Muro, Y. and Himeno, M. (1996) Human homolog of *Drosophila* heterochromatin-associated protein 1 (HP1) is a DNA-binding protein which possesses a DNA-binding motif with weak similarity to that of human centromere protein C (CENP-C). *J. Biochem.* (Tokyo), **120**, 153–159.

Taddei, A., Maison, C., Roche, D. and Almouzni, G. (2001) Reversible disruption of pericentric heterochromatin and centromere function by inhibiting deacetylases. *Nat. Cell Biol.*, **3**, 114–120.

Weiss, M.A. and Narayana, N. (1998) RNA recognition by arginine-rich peptide motifs. *Biopolymers*, **48**, 167–180.

Zhao, T., Heyduk, T., Allis, C.D. and Eissenberg, J.C. (2000) Heterochromatin protein 1 binds to nucleosomes and DNA *in vitro*. *J. Biol. Chem.*, **275**, 28332–28338.

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